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Risk and Clearance of GB Virus C/Hepatitis G Virus Infection in Homosexual Men: A Longitudinal Study

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The risk and clearance of GB virus type C (GBV-C)/hepatitis G virus (HGV) infection was investigated in a cohort of homosexual men (n=180; median follow-up time, 7 years). The interaction between GBV-C/HGV RNA and antibodies against the E2 region of the virus, and the clinical impact of chronic GBV-C/HGV infection were studied. GBV-C/HGV RNA was detected by RT-PCR, and E2 antibodies were assessed by an immunoassay. At baseline, 63% of the participants had evidence of previous or current GBV-C/HGV infection. The GBV-C/HGV incidence rate was 2 per 100 person-years (95% confidence interval 0.9–3.8) and was similar to the HIV incidence. The incidence of GBV-C/HGV infection was significantly higher in those reporting unprotected anal intercourse (3.6 per 100 person-years compared to 0 in the group without such sexual contacts). The occurrence of E2 antibodies was strongly associated with GBV-C/HGV RNA clearance. A loss of E2 antibodies was observed at a rate of 1.5 per 100 person-years. It was higher among HIV-infected individuals. Chronic GBV-C/HGV infection was not associated with clinical or biochemical evidence of liver disease. ***J. Med. Virol. 59:303–306, 1999.***

KEY WORDS: GBV-C/HGV; incidence; viral clearance; sexual transmission; hepatitis

INTRODUCTION

GB virus type C (GBV-C)/hepatitis G virus (HGV) is transmitted readily by the parenteral route [Simons et al., 1995; Linnen et al., 1996; Jarvis et al., 1996; Schreier et al., 1996; Feucht et al., 1997; Thomas et al., 1997]. A few cross-sectional studies on GBV-C/HGV RNA prevalence among homosexual men who were not injecting drug users, or individuals with sexually-acquired HIV infection suggested that sexual transmission may also play a role [Stark et al., 1996; Fiordalisi et al., 1997; Rubio et al., 1997; Nerurkar et al., 1998]. Studies based on both GBV-C/HGV RNA and GBV-C/HGV-E2 antibody detection by immunoassay reported high overall GBV-C/HGV infection prevalences of up to 10% among non-remunerated blood donors [Pilot-Matias et al., 1996; Tacke et al., 1997]. One possible explanation for this finding is sexual transmission. However, longitudinal studies which quantify the risk of sexual transmission are not available.

In cross-sectional studies, the presence of both GBV-C/HGV RNA and anti-E2 was observed rarely [Stark et al., 1997; Tacke et al., 1997; Thomas et al., 1998]. The virus appears to be cleared in many infected individuals, although viral persistence for more than 10 years has been reported in isolated cases [Thomas et al., 1997; Alter et al., 1997]. There is some evidence that the occurrence of GBV-C/HGV-E2 antibodies is associated with viral clearance, based on a few longitudinal studies among parenterally exposed populations [Thomas et al., 1998]. It is unclear, however, if the presence of E2 antibodies confers long-lasting immunity.

The possible causative role of GBV-C/HGV infection in fulminant hepatitis or in chronic hepatitis non-A-E is still a matter of controversy. Again, the data from longitudinal studies are scarce [Alter et al., 1997; Mushahwar and Zuckerman, 1998].

We conducted a study among a cohort of homosexual men who were not injecting drug users. The objectives of the study were to determine the risk of new GBV-C/HGV infections, the GBV-C/HGV clearance rate, the incidence and possible loss of GBV-C/HGV-E2 antibodies, and the association between GBV-C/HGV RNA and anti-E2 in the course of the infection. In addition, we

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investigated whether GBV-C/HGV infection was associated with biochemical evidence of liver damage.

MATERIAL AND METHODS

Study Population

The study population (n = 180) was selected randomly from of a cohort of homosexual men in Germany. This cohort had been set up in 1985 to investigate the risk factors and the natural history of human immunodeficiency virus (HIV) infection [Schwartländer et al., 1993]. A total of 780 individuals were recruited and followed over a median time of 7 years (interquartile range 5.5 to 8.5 years). The baseline HIV prevalence was 54%. Laboratory tests were undertaken for a variety of markers (serological and biochemical markers). The participants were interviewed for medical history as well as demographic and behavioural characteristics. The participants were invited for follow-up investigations every 6 months. At each follow-up, serum aliquots were stored at −70°C. The stored sera were tested for GBV-C/HGV RNA, GBV-C/HGV-E2 antibodies, and hepatitis C virus (HCV) antibodies. The results of tests for HIV antibodies and hepatitis B virus (HBV) seromarkers were available from the original study.

Detection of GBV-C/HGV RNA

GBV-C/HGV RNA was determined as described previously [Schreier et al., 1996]. RNA was extracted from 140 μl serum by spin column technique (Qiagen, Hilden, Germany) and resolved in 50 μl water; 2.5 μl of the RNA preparation were reverse transcribed using 50 U of M-MLV reverse transcriptase according to the manufacturer's instructions (GIBCO BRL Life Technologies, Inc., Gaithersburg, MD) and primer G9 5′-TCYTTGATGATDGAACTGTC-3′ for the NS3 region [Yoshiba et al., 1995] and primer E2-1 5′-GCGGGTCGCCAAATCCCCAT-3′ for the E2 region. One fourth of the cDNA was amplified by 35 cycles of PCR (94°C 30 seconds, 42 or 45°C 30 seconds, and 72°C 45 seconds) using primers G9 and NS3-2 5′-CGAGGCAACCCGGTGCGGTCA-3′ for the NS3 region and primers E2-1 and E2-2 5′-GAGATTCCCTTTTATGGGCATGG-3′ for the E2 region. The second round PCR was carried out under the same conditions with primers G11 [Yoshiba et al., 1995] 5′-TCYTTACCCCTRTAATAGGC-3′ and NS3-4 5′-ATACCCCTCGAGMGGATGCG-3′for the NS3 region and primers E2-3 5′-CTTCCACAACACGAGGAACAT-3′ and E2-4 5′-CTTGGGTTTTGGGTCCTAC-3′ for the E2 region. The PCR products were sequenced directly using the ABI Prism 377 DNA sequencer and a dye terminator cycle sequencing kit (Perkin-Elmer, Norwalk, CT) as recommended by the manufacturer. The data were analysed using the program "Sequence Navigator" (Applied Biosystems, Foster City, CA). In 12 randomly-selected individuals who were GBV-C/HGV RNA positive at baseline and at the last follow-up, we examined the genetic drift of GBV-C/HGV. A 423 nt long fragment of the E2 region (1884-2306 nt position in HGV PNF2161) was amplified and sequenced.

TABLE I. GBV-C/HGV Seromarkers in a Cohort of Homosexual Men (Median Follow-Up Period 7 Years)

Number of individuals with GBV-C markers at	
Baseline	Last follow-up visit
48 GBV-C RNA+	36 GBV-C RNA+/anti-E2–
(47 anti-E2–, 1 anti-E2+)	3 GBV-C RNA+/anti-E2+
	8 GBV-C RNA–/anti-E2+
	1 GBV-C RNA–/anti-E2–
67 GBV-C RNA–/anti-E2–	58 GBV-C RNA–/anti-E2–
	3 GBV-C RNA+/anti-E2–
	1 GBV-C RNA+/anti-E2+
	5 GBV-C RNA–/anti-E2+
65 GBV-C RNA–/anti-E2+	58 GBV-C RNA–/anti-E2+
	7 GBV-C RNA–/anti-E2–

Antibodies Against GBV-C/HGV-E2, HIV, HBV, and HCV

Antibodies against the E2 protein of GBV-C/HGV were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (Boehringer Mannheim, Germany) [Tacke et al., 1997]. An ELISA (Abbott, Wiesbaden, Germany) was used to identify antibodies against HIV and HCV, and seromarkers for HBV infection. Positive results were confirmed by Western Blot (HIV) and RIBA (HCV).

Statistical Analysis

Associations between categorical variables were assessed by chi² tests. Incidence rates for GBV-C/HGV, HBV, HCV, and HIV infection were calculated per 100 person-years, and the respective 95% confidence interval (CI) were calculated by the exact method based on the Poisson distribution. The date of seroconversion was estimated as the midpoint between the last negative and the first positive result.

RESULTS

Prevalence of GBV-C/HGV RNA and Anti-E2

Of the 180 participants (median age 35 years, interquartile range 28 to 43 years), 48 individuals (27%) were positive for GBV-C/HGV RNA at baseline, and 66 (37%) were anti-E2 positive. In only one individual were both markers present. Thus, the overall prevalence of current or previous GBV-C/HGV infection was 63%. The seroprevalences for the other infectious agents were 52% for HIV, 62% for HBV (vaccinated 26%), and 2% for HCV. GBV-C/HGV infection was not significantly associated with age, HIV, HBV, or HCV infection.

Incidence of GBV-C/HGV Infection

Of the 67 participants who were seronegative for both GBV-C/HGV RNA and anti-E2 at baseline, 9 had serological evidence for a newly-acquired GBV-C/HGV infection at the end of the follow-up period (Table I). Five of these converters developed E2 antibodies, but no GBV-C/HGV RNA was detectable. In these individuals the follow-up intervals between the first positive and the last negative anti-E2 result lasted between 1

TABLE II. Seroconversion Rates for GBV-C/HGV, HIV, HBV, and HCV Infection Among Homosexual Men

Virus	Number of individuals at risk at baseline	Number of sero-conversions	Incidence rate (per 100 person-years)	95% CI[a]
GBV-C/HGV (n = 180)	67	9	2.0	0.9–3.8
HIV (n = 178)	85	12	2.0	1.0–3.4
HBV (n = 126)	13	2	2.3	0.3–8.2
HCV (n = 178)	175	2	0.1	0.02–0.5

[a]CI, confidence interval.

and 2 years, so that shorter periods of GBV-C/HGV viremia could have been missed. The overall GBV-C/HGV incidence rate was 2 per 100 person-years (95% CI 0.9-3.8) (Table II).

None of the participants who were anti-E2 positive at baseline or at any later time became GBV-C/HGV RNA positive. All nine converters reported unprotected receptive or insertive anal intercourse (eight individuals with more than one partner) in the year before GBV-C/HGV RNA conversion whereas only 31% of the non-converters reported such contacts during a comparable time period ($P<0.001$). The incidence rate was 3.6 per 100 person-years (95% CI 1.7–6.8) among the group (n = 37) reporting unprotected sexual contacts at any time during the follow-up period and 0 among the group (n = 30) without such contacts ($P<0.01$). GBV-C/HGV seroconversion was not associated with age, HIV, or HBV serostatus.

Clearance of GBV-C/HGV RNA

Of the 48 participants initially positive for GBV-C/HGV RNA, 9 cleared the virus (clearance rate 2.7 per 100 person-years, 95% CI 1.2–5.1). Eight of these individuals had developed E2 antibodies by the end of the follow-up period. Except for one individual where GBV-C/HGV RNA and anti-E2 co-persisted for 2.5 years, these individuals cleared GBV-C/HGV RNA a few months after having developed antibodies.

Loss of GBV-C/HGV-E2 Antibodies

Of the 66 individuals who were anti-E2 positive at baseline, 7 lost the antibodies during the follow-up period, at a rate of 1.5 per 100 person-years (95% CI 0.6–3.0). There was a trend that more HIV-infected individuals lost their E2 antibodies (6 out of 38) than HIV-negative individuals (1 out of 27) ($P = 0.1$). This finding is not due to differences in the follow-up among these two groups.

GBV-C/HGV Infection and ALT Levels

At baseline, the levels of alanine aminotransferase (ALT) were significantly elevated (>45 IU/l) in 2% of the GBV-C/HGV RNA negative individuals, and none of the GBV-C/HGV RNA positive individuals. Self-limiting ALT elevations shortly before or after GBV-C/HGV seroconversion occurred in 4 of the 9 GBV-C/HGV converters (maximum ALT 89 IU/ml). Persistent GBV-C/HGV viremia was not associated with increased ALT levels.

Variability of the E2 Region

In 12 participants persistently positive for GBV-C/HGV RNA (6 were also anti-HIV-positive), the first and the last sample was examined (median follow-up 8 years) for genetic drift in the GBV-C/HGV-E2 region (detailed results available from the authors). Four participants developed anti-E2 antibodies. The estimated nucleotide substitution rate was low (between 0 and 3.2×10^{-4} substitutions per genome site per year, i.e., an amino acid homology between 92 and 99%), and was independent of the HIV status of the individuals and the occurrence of E2 antibodies.

DISCUSSION

In this cohort of homosexual men, the baseline GBV-C/HGV infection prevalence based on the detection of GBV-C/HGV RNA or anti-E2 was high. A few other studies reported somewhat lower GBV-C/HGV prevalences among homosexuals [Stark et al., 1996; Nübling et al., 1997; Nerurkar et al., 1998]. These data provide evidence for sexual transmission of GBV-C/HGV but do not allow to quantify the incidence of infection. The relatively high prevalence in our study at baseline may be due to the sampling of a high-risk population according to the original study design of the AIDS cohort study.

An GBV-C/HGV seroincidence of 2 per 100 person-years was observed among homosexual men who have not been exposed to other known risk factors of GBV-C/HGV infection. Seroconversion was associated significantly with unprotected anal sex. Although based on a relatively low number of seroconverters, these data clearly demonstrate that GBV-C/HGV is transmitted not only via contaminated blood or blood products but by sexual contact as well. This is supported by a recent study that detected GBV-C/HGV RNA in the semen of 50% of GBV-C/HGV seropositive individuals [Semprini et al., 1998]. It is most likely that GBV-C/HGV transmission in our study occurred via anal sexual intercourse. However, at present other routes of transmission by intimate contact (e.g., oral sex) cannot be ruled out entirely. Larger epidemiological studies are needed to elucidate further any specific risk factors for non-parenteral GBV-C/HGV infections.

Our study suggests that the rates of sexual transmission among homosexual men are similar for GBV-C/HGV and HIV infection. However, also taking into account the results from parenterally exposed cohorts (e.g., injecting drug users) [Thomas et al., 1998] paren-

teral transmission of GBV-C/HGV appears to be more likely than sexual transmission.

At baseline, both GBV-C/HGV RNA and anti-E2 were detected in only one individual. However, during the follow-up period GBV-C/HGV RNA and anti-E2 co-persisted for some time in a few individuals. In most of these cases, GBV-C/HGV RNA disappeared a few months after the detection of anti-E2. This corroborates previous reports by ourselves and others from studies among injecting drug users [Tacke et al., 1997; Thomas et al., 1998] that the development of E2 antibodies is associated with viral clearance. In our cohort, no re-infections were observed once antibodies had developed. Taken together, these results provide strong evidence that the GBV-C/HGV-E2 antibodies are protective. In this respect, GBV-C/HGV infection seems to resemble HBV infection, but is in contrast to HCV or HIV infection.

In many viral infections (e.g., hepatitis B or C), antibodies tend to persist for long periods or throughout life. In contrast, in GBV-C/HGV infection the loss of antibodies is not an uncommon event. There was a trend that HIV-infected individuals were more likely to loose GBV-C/HGV antibodies than HIV-negative individuals. In another study this difference was statistically significant [Devereux et al., 1998]. The immunodeficiency caused by HIV infection may result in a more rapid decline in E2 antibody concentrations as has been observed for anti-HBs or anti-HCV antibodies.

In 39 participants of the current study, GBV-C/HGV RNA persisted throughout the whole follow-up period. The nucleotide substitution rate was very much lower than substitution rates in persistent HCV infection [Kato et al., 1998]. The finding that the nucleotide substitution rates did not differ between individuals with GBV-C/HGV-E2 antibody response and those without one does not suggest the occurrence of escape mutants. Larger studies including additional GBV-C/HGV regions are necessary to investigate why some individuals remain GBV-C/HGV RNA positive for many years and why GBV-C/HGV RNA and anti-E2 antibodies may co-persist for up to two or three years in isolated cases.

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